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Running title: Exercise-induced reduction in food intake

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Abstract

Background

Repetitive physical activity is a well-established intervention to reduce obesity and to prevent weight regain. Besides increased energy expenditure, reduced caloric intake may contribute to exercise-induced weight loss in obesity. Using adipocyte-specific glycoprotein 130 knockout ($gp130^{\Delta adipo}$) mice, we recently unravelled that obesity-induced interleukin-6 (IL-6) signalling in adipose tissue contributes to circulating levels of the two anorectic hormones leptin and insulin. Herein, we aimed to investigate the role of adipocyte-specific IL-6 signalling in exercise-mediated appetite control and, hence, weight reduction in obesity.

Methods

$gp130^{\Delta adipo}$ and control littermate mice ($gp130^{F/F}$) were repetitively exercised during a 12-week period of HFD-feeding. Thermogenesis was determined using thermography and food intake as well as energy expenditure were assessed in metabolic cages. Circulating IL-6, insulin and leptin levels were measured using immunoassays. Protein levels of phosphorylated STAT3, JAK2 and Akt were determined in the hypothalamus by Western blot technique.

Results

Repetitive physical activity reduced food intake and HFD-induced weight gain in $gp130^{F/F}$ but not $gp130^{\Delta adipo}$ mice. In contrast, energy expenditure was not different between the genotypes. Circulating insulin and leptin levels were significantly reduced in

gp130$^{\Delta adipo}$ mice. Moreover, hypothalamic leptin and insulin signalling was enhanced in exercised gp130$^{F/F}$ but not gp130$^{\Delta adipo}$ mice as demonstrated by elevated pSTAT3, pJAK2 and pAkt protein levels.

Conclusion

Adipocyte-specific IL-6 signalling is involved in exercise-mediated regulation of food intake and weight reduction in HFD-fed mice.

Introduction

Repetitive physical activity results in reduced body weight and blunted insulin resistance in obese human subjects (1, 2). Importantly, increased physical activity may not only increase energy expenditure but also decrease food intake in obese humans (3, 4), indicating that reduced caloric intake contributes to exercise-induced weight loss. Moreover, exercise-mediated appetite control in obese subjects may contribute to its well-established efficacy in the prevention of weight regain (4). However, underlying molecular mechanisms remain incompletely understood.

Interleukin-6 (IL-6) acts as a physiological regulator of energy homeostasis during exercise. IL-6 release from skeletal muscle increases during physical activity, and this IL-6 increase is thought to mobilize free fatty acids (FFAs) from white adipose tissue as a fuel source (5). Besides affecting FFA release, IL-6 was shown to induce leptin production from adipose tissue *ex vivo* (6). In line, we recently demonstrated that obesity-augmented IL-6 release induces leptin secretion from adipocytes in mice, thereby enhancing insulin secretion (7, 8). In fact, high fat diet (HFD)-fed adipocyte-specific glycoprotein 130 knockout ($gp130^{\Delta adipo}$) mice revealed significantly reduced circulating leptin levels (7). Consequently, leptin-mediated release of glucagon-like peptide-1 (GLP-1) from enteroendocrine cells was reduced, leading to blunted glucose-stimulated insulin release. gp130 is a signal transducer protein of the IL-6 signalling pathway (9). After binding of IL-6 to its receptor, this complex interacts with a gp130 homodimer to initiate its signalling. Leptin is mainly produced and secreted by white adipocytes. It is a key hormone in the regulation of body weight as it decreases food intake and increases energy expenditure (10-13). Besides leptin, insulin induces satiety in the hypothalamus (14). In obesity, emerging insulin and leptin resistance may

contribute to weight gain (10, 14). In line, reduced circulating leptin and insulin levels in HFD-fed gp130$^{\Delta adipo}$ mice did not affect food intake and body weight (7), suggesting that evolved central insulin and leptin resistance may have masked the difference in the former. Accordingly, improving central insulin and/or leptin sensitivity by repetitive physical activity (15, 16) may unmask the difference in circulating insulin and leptin levels and, thus, may reduce food intake in HFD-fed gp130$^{F/F}$ but not in gp130$^{\Delta adipo}$ mice. Herein, we hypothesized that adipocyte-specific IL-6 signalling is involved in exercise-mediated reduction in food intake and, consequently, weight reduction in obese mice.

Materials and Methods

Animals

Adipocyte-specific gp130 knockout mice ($gp130^{\Delta adipo}$) on a C57BL/6J background were generated by crossing gp130 floxed ($gp130^{F/F}$) mice (17) with animals expressing the Cre recombinase controlled by the Adipoq promoter (AdipoqCre mice; purchased from The Jackson Laboratory, Bar Harbor, ME, USA). Six weeks-old male mice were fed *ad libitum* with high fat diet (HFD; Surwit with sucrose, ssniff-Spezialdiäten GmbH, Soest, Germany) for 12 weeks. HFD consisted of 59% of calories derived from fat, 26% from carbohydrate and 15% from protein. All protocols conformed to the Swiss animal protection laws and were approved by the Cantonal Veterinary Office in Zurich, Switzerland. No randomization was used to allocate animals to intervention groups. The investigator was blinded to the identity of the mice as far as the nature of the experiment allowed it.

Intraperitoneal insulin tolerance test

Intraperitoneal insulin tolerance tests were performed as described previously (18). Blood glucose concentration was measured in blood received after tail vessel incision using a glucometer (AccuCheck Aviva, Roche Diagnostics, Rotkreuz, Switzerland).

Food intake and indirect calorimetry

To assess acute food intake after a bout of exercise, mice were single caged for 3 hours and food was weighed before and after. Chronic food intake and indirect calorimetry was determined using a metabolic and behavioural monitoring system

(PhenoMaster, TSE Systems, Bad Homburg, Germany) as described (19). Data were recorded during a 48-hour period. Average of the two days was calculated.

Exercise training

Exercise training was initiated at the onset of the HFD at the age of 6 weeks. Mice ran on a treadmill for 60 minutes/day, 5 days/week. To adapt mice to the treadmill, speed was step wise increased during the first 4 days (10 cm/s for 1 min, 13 cm/s for 3 min and 15 cm/s for 56 min). Thereafter, treadmill speed was set at a constant rate (17 cm/s) for the 60 min training period, and speed was increased by 1 cm/s every 5th day until reaching the final speed of 20 cm/s. Mice were motivated to run using a tongue depressor as previously described (20).

Rectal temperature measurement

A digital thermometer (ama-digit ad 15th, Amarell GmbH, Kreuzwertheim, Germany) was used in combination with a stainless probe. The probe was inserted 2 cm into the anal duct of mice. Temperature was measured before and after a bout of exercise.

Thermography

Thermal imaging was performed using the infrared camera Fluke Ti45 IR Flexcam Thermal Imager (Fluke Europe B.V., Eindhoven, Netherlands) with a 20 mm objective (F/0.8 8-14 µm JTI-40948-4937) processing mostly 8 to 14 µ infrared emissions. The camera was mounted onto a mobile frame allowing pictures to be taken vertically from 50 cm above the running field. The accuracy of the temperature measurement was ± 0.7ºC. The camera was adjusted to a calibrated external

thermometer before and after measurements and remained constant during application. Pictures were taken from two mice (one gp130$^{F/F}$ and one gp130$^{\Delta adipo}$) running separately in a race course for 60 minutes. Room temperature remained in the range of 24 ± 0.5ºC during the experiment. Temperature profiles were analysed with Fluke SmartView® software.

Blood sampling

Blood was collected from tail vein after incision with a razor blade. EDTA (5mM) was added to the collected blood followed by immediate centrifugation at 4°C. Thereafter, plasma was stored at -80°C until further processing.

Determination of plasma IL-6, insulin and leptin

Plasma IL-6 and leptin levels were determined using MSD technology. Insulin was assessed using an ELISA kit as described (21).

Western blotting

Hypothalamus as well as brown adipose tissue samples were homogenized as described previously (22). Protein concentration was determined using BCA assay (Pierce, Rockford, IL, USA) and equivalent amounts of protein were resolved by SDS-PAGE (BioRad Laboratories, Cressier, Switzerland). Proteins were electro-transferred onto nitrocellulose membranes (0.2 µm, BioRad Laboratories) and immunoblotted for anti-UCP1 (PA1-24894, Thermo Fisher Scientific, Waltham, MA, USA), anti-phospho-STAT3 (9145, Cell Signaling Technology, Danvers, MA, USA), anti-STAT3 (9132, Cell Signaling Technology), anti-phospho-JAK2 (3776, Cell Signaling Technology), anti-JAK2

(sc-390539, Santa Cruz Biotechnology, Heidelberg, Germany), anti-phospho-Akt (4058, Cell Signaling Technology), anti-Akt (9272, Cell Signaling Technology) or anti-actin (MAB 1501, Merck, Schaffhausen, Switzerland). Membranes were exposed and analysed using the ChemiDoc Imaging System (BioRad Laboratories).

Data analysis

Data are presented as means ± SEM. Data were analysed by unpaired two-tailed Student's *t* test or two-way ANOVA with Bonferroni multiple comparisons. Welch's correction was used for data with unequal variances. All statistical tests were calculated using the GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA). P values < 0.05 were considered to be statistically significant. Sample size was determined based on previous animal experiments performed in our laboratory. Data differing more than +/- 2SD from the mean were excluded.

Results

Exercise reduces HFD-induced weight gain in gp130$^{F/F}$ but not gp130$^{\Delta adipo}$ mice

To investigate whether adipocyte-specific IL-6-type cytokine signalling affects exercise-induced weight reduction, adipocyte-specific gp130 knockout mice (gp130$^{\Delta adipo}$) and control littermate mice (gp130$^{F/F}$) were repetitively exercised (Ex) during a 12-week period of HFD-feeding. Adipocyte-specific depletion of gp130 in gp130$^{\Delta adipo}$ mice was recently confirmed (23). Exercise training was initiated at the onset of HFD at the age of 6 weeks and mice ran on a treadmill for 60 minutes/day, 5 days/week (24). As sedentary (Sed) controls, HFD-fed gp130$^{F/F}$ and gp130$^{\Delta adipo}$ mice were handled 5 days/week without undergoing exercise training (Fig. 1A). As expected, circulating IL-6 levels increased after a bout of exercise in both genotypes (Fig. 1B). As intended (16), repetitive physical exercise reduced HFD-induced body weight gain in gp130$^{F/F}$ mice (Fig. 1C). In contrast, such effect was blunted in gp130$^{\Delta adipo}$ mice (Fig. 1D), suggesting that IL-6 signalling in adipocytes plays an important role in exercise-induced body weight reduction in obesity. To analyse whether adipocyte-specific gp130 signalling is involved in the positive effect of repetitive physical activity in ameliorating obesity-induced insulin resistance, intraperitoneal insulin tolerance tests were performed. As previously reported (16), exercise training improved insulin sensitivity in HFD-fed gp130$^{F/F}$ mice (Fig. 1E). In contrast, such effect was blunted in HFD-fed gp130$^{\Delta adipo}$ mice (Fig. 1F). Hence, adipocyte-specific gp130 signalling contributes to the insulin-sensitizing effect of repetitive physical activity.

Next, acute food intake following a bout of exercise was analysed. Compared to sedentary mice, exercise reduced food intake by ~60% in HFD-fed gp130$^{F/F}$ but only by ~20% in gp130$^{\Delta adipo}$ (Fig. 1G) mice. Hence, IL-6 signalling in adipocytes may mediate

exercise-induced reduction in food intake in HFD-fed mice. Although not statistically significant, exercised gp130$^{F/F}$ mice consumed ~50% less food compared to exercised gp130$^{\Delta adipo}$ mice (gp130$^{F/F}$ 0.07±0.03 g vs. gp130$^{\Delta adipo}$ 0.16±0.07) within a 3-hour period following a bout of exercise.

Adipocyte-specific IL-6 signalling affects food intake but not energy expenditure after repetitive physical activity

To analyse food intake during a 48-hour period and to assess energy expenditure, mice were put into metabolic cages after 9 weeks of HFD/exercise training. As shown in Fig. 2A, food intake was significantly increased in exercised gp130$^{\Delta adipo}$ compared to gp130$^{F/F}$ mice, further supporting an important role of adipocyte-specific gp130 signalling in exercise-mediated regulation of food intake. Respiratory quotient was significantly lower in exercised compared to sedentary gp130$^{F/F}$ mice (Fig. 2B), indicating that exercised gp130$^{F/F}$ mice oxidize more fat. In contrast, energy expenditure was similar between the groups (Fig. 2C). To analyse acute thermogenesis, body surface temperature was assessed during exercise as well as rectal temperature before and after a bout of exercise. Rectal temperature did not change neither before nor after physical exercise in gp130$^{\Delta adipo}$ mice (data not shown). As expected, body surface temperature increased in both genotypes during exercise, however to a similar degree as depicted in Figs. 3A and 3B. In line, uncoupling protein 1 (UCP1) levels in brown adipose tissue (BAT) were similar between exercised gp130$^{F/F}$ and gp130$^{\Delta adipo}$ mice (Fig. 3C), indicating that reduced leptin levels does not seem to affect thermogenesis in BAT (25). Similarly, there was no significant difference in UCP1 protein levels between sedentary gp130$^{F/F}$ and gp130$^{\Delta adipo}$ mice (Fig. 3C). Taken together, adipocyte-specific

gp130 signalling affects food intake but not energy expenditure and thermogenesis in exercised HFD-fed mice.

Enhanced leptin and insulin sensitivity in HFD-fed gp130$^{F/F}$ but not gp130$^{\Delta adipo}$ mice

To investigate whether insulin and/or leptin may contribute to reduced food intake in exercised HFD-fed gp130$^{F/F}$ mice (Figs. 1G and 2A), their circulating levels and hypothalamic sensitivity to these hormones were determined. Plasma leptin and insulin levels were significantly lower in exercised gp130$^{\Delta adipo}$ compared to gp130$^{F/F}$ mice (Figs. 4A and 4B). In agreement, non-exercised adipocyte-specific gp130 knockout mice revealed reduced circulating levels of these two anorectic hormones in HFD-fed mice (7). Next, leptin and insulin signalling in the hypothalamus was assessed using Western blot technique. Of note, repetitive exercise was previously reported to improve central leptin signalling in HFD-fed mice (16). In particular, it increased hypothalamic JAK2 and STAT3 phosphorylation in obese rodents (26). As depicted in Figs. 4C to 4F, exercise improved phosphorylation of JAK2 and STAT3 in exercised gp130$^{F/F}$ but not in exercised gp130$^{\Delta adipo}$ mice. In addition, hypothalamic Akt phosphorylation was improved in gp130$^{F/F}$ mice after repetitive physical activity, indicating elevated insulin sensitivity in the latter. Of note, protein levels of total JAK2, STAT3 and Akt were significantly elevated in exercised gp130$^{F/F}$ but not in exercised gp130$^{\Delta adipo}$ mice (Figs. 4C to 4F). These data indicate that elevated hypothalamic insulin and leptin signalling in gp130$^{F/F}$ mice contribute to decreased food intake after exercise. Importantly and as expected, gp130 protein levels were similarly expressed in the hypothalamus of gp130$^{F/F}$ and gp130$^{\Delta adipo}$ mice (data not shown), further supporting adipocyte-specific expression of Cre in the AdipoqCre mouse.

Discussion

The present study suggests that IL-6 signalling in adipocytes is critically involved in exercise-mediated regulation of food intake and, consequently, body weight gain in HFD-fed mice (Fig. 5). Mechanistically, such effect may depend on: 1) HFD-induced IL-6-signalling in adipocytes resulting in increased leptin and insulin release. Latter may be induced by leptin-mediated release of glucagon-like peptide-1 (GLP-1) from enteroendocrine cells (7). 2) exercise-induced increase in hypothalamic insulin and leptin signalling, which was previously reported to be improved in exercised HFD-fed rodents (10, 15, 16, 26). Of note, elevated signalling may be mediated by increased circulating levels of leptin and insulin and/or by increased protein levels of key mediators of the respective signaling cascades such as JAK2, STAT3 or Akt. These findings suggest that lipid loaded adipocytes communicate with the hypothalamus to reduce food intake after exercise. Physiologically, such crosstalk is meaningful since exercise-induced energy demand may be covered by stored rather than newly consumed calories in the obese state.

In line with our findings, exercise reduced food intake in obese rats as well as in ob/ob mice (26). In the latter study, hypothalamic IL-6 signalling was required for the exercise-mediated reduction in food intake in obese rats. As circulating IL-6 levels and hypothalamic gp130 protein levels were similar in $gp130^{F/F}$ and $gp130^{\Delta adipo}$ mice, hypothalamic IL-6 signalling may be functional in $gp130^{\Delta adipo}$ mice, suggesting that IL-6 signalling in the hypothalamus may not contribute to the observed phenotype. In contrast, insulin and leptin signalling may be critically involved in exercise-mediated inhibition of food intake and body weight gain in HFD-fed $gp130^{F/F}$ mice. In fact,

circulating leptin and insulin levels were increased in exercised HFD-fed gp130$^{F/F}$ compared to gp130$^{\Delta adipo}$ mice resulting in elevated hypothalamic leptin and insulin signalling in the first.

Besides modulating food intake, leptin may affect energy expenditure and thermogenesis in BAT (13, 25). However, we found no difference in energy expenditure, thermogenesis or UCP1 protein levels in BAT between gp130$^{F/F}$ and gp130$^{\Delta adipo}$ mice. Possibly, the observed difference in circulating leptin concentration between the two genotypes was too little to influence aforementioned parameters. Alternatively, high fat diet feeding may have induced leptin resistance in BAT that was not reversed by repetitive physical activity.

Based on the finding that exercise decreased respiratory quotient in gp130$^{F/F}$ but not in gp130$^{\Delta adipo}$ mice, adipocyte-specific depletion of the IL-6 signalling pathway blunts exercise-induced incrase in fat oxidation. These data may indicate that IL-6-induced mobilization of FFA from white adipose tissue (5) is blunted in gp130$^{\Delta adipo}$ mice. In line, IL-6 knockout (KO) mice revealed increased respiratory quotient (27), supporting a role of IL-6 in the regulation of lipid oxidation. Besides, IL-6 KO mice revealed impaired exercise endurance capacity, as measured by running time to exhaustion. While exercise endurance was not assessed in the present study, we did not observe any difference in exercise capacity at the chosen submaximal exercise level between HFD-fed gp130$^{F/F}$ and gp130$^{\Delta adipo}$ mice. All mice of both genotypes completed the planned exercise regimen and frequency of motivation stimuli was similar between the genotypes (data not shown).

Herein, we confirm a positive effect of exercise on glucose metabolism. In fact, insulin sensitivity was significantly improved in HFD-fed mice undergoing repetitive

physical activity. Importantly, such effect was clearly blunted in mice with adipocyte-specific depletion of gp130. Hence, IL-6-type cytokine signalling in adipocytes is involved in the ameliorating effect of exercise on HFD-induced insulin resistance. Blunted exercise-induced reduction in body weight gain in gp130$^{\Delta adipo}$ mice may contribute to such effect.

In conclusion, adipocyte-specific IL-6 signalling is involved in exercise-mediated reduction in food intake and, consequently, body weight gain in HFD-fed mice. As exercise-mediated appetite control in obesity is not only important for weight reduction but also for the prevention of weight regain, the herein identified pathway may be of importance for body weight management.

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Competing Interests

All authors state no conflict of interest.

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Figure Legends

Figure 1 Exercise reduces HFD-induced weight gain in gp130$^{F/F}$ but not gp130$^{\Delta adipo}$ mice

(**A**) Study design. (**B**) Circulating IL-6 levels were assessed in HFD-fed exercised gp130$^{F/F}$ and gp130$^{\Delta adipo}$ mice (n=7). Blood was sampled immediately before and after a bout of exercise after 7 weeks of exercise training. (**C**) Weight gain in HFD-fed exercised (Ex; n=8) and sedentary (Sed; n=5) gp130$^{F/F}$ mice. (**D**) Weight gain in HFD-fed exercised (Ex; n=8) and sedentary (Sed; n=6) gp130$^{\Delta adipo}$ mice. (**E**) Insulin tolerance (ipITT) tests were performed in HFD-fed exercised (Ex; n=7) and sedentary (Sed; n=5) gp130$^{F/F}$ mice. (**F**) ipITT were performed in HFD-fed exercised (Ex; n=8) and sedentary (Sed; n=5) gp130$^{\Delta adipo}$ mice. (**G**) Food intake was determined in high fat diet-fed exercised (Ex; n=6) and sedentary (Sed; n=4) gp130$^{F/F}$ mice as well as in high fat diet-fed exercised (Ex; n=5) and sedentary (Sed; n=5) gp130$^{\Delta adipo}$ mice during a 3-hour period following a bout of exercise after 11 weeks of exercise/HFD. Values are expressed as mean ± SEM. *p < 0.05, **p < 0.01. Statistical tests used: *t*-tests for B and E (AUC); two-way ANOVA for C and E. AUC: area under the curve; F/F: gp130$^{F/F}$; ∆ad: gp130$^{\Delta adipo}$.

Figure 2 Adipocyte-specific IL-6 signalling affects food intake but not energy expenditure after repetitive physical activity

Food intake (**A**), respiratory quotient (**B**) and energy expenditure (**C**) were assessed in HFD-fed exercised (Ex; n=4) and sedentary (Sed; n=9) gp130$^{F/F}$ as well as exercised (Ex; n=4) and sedentary (Sed; n=7) gp130$^{\Delta adipo}$ mice after 9 weeks of exercise/HFD.

Values are expressed as mean ± SEM. *p < 0.05 (ANOVA). F/F: gp130$^{F/F}$; ∆ad: gp130$^{\Delta adip}$.

Figure 3 Similar thermogenesis between HFD-fed exercised gp130$^{F/F}$ and gp130$^{\Delta adipo}$ mice

Representative images (**A**) and quantification (**B**) of thermographic analyses in HFD-fed exercised (Ex) gp130$^{F/F}$ (n=6) and gp130$^{\Delta adipo}$ (n=6) mice after 10 weeks of exercise training. A and B in Fig. 3A correspond to time point 0 min (A) and 60 min (B) in Fig. 3B. (**C**) Representative Western blot and quantified protein levels of UCP1 in total lysates of brown adipose tissue harvested from HFD-fed exercised (Ex, n=7) and sedentary (Sed; n=5) gp130$^{F/F}$ as well as exercised (Ex, n=8) and sedentary (Sed; n=6) gp130$^{\Delta adipo}$ mice. Values are expressed as mean ± SEM. F/F: gp130$^{F/F}$; ∆ad: gp130$^{\Delta adipo}$.

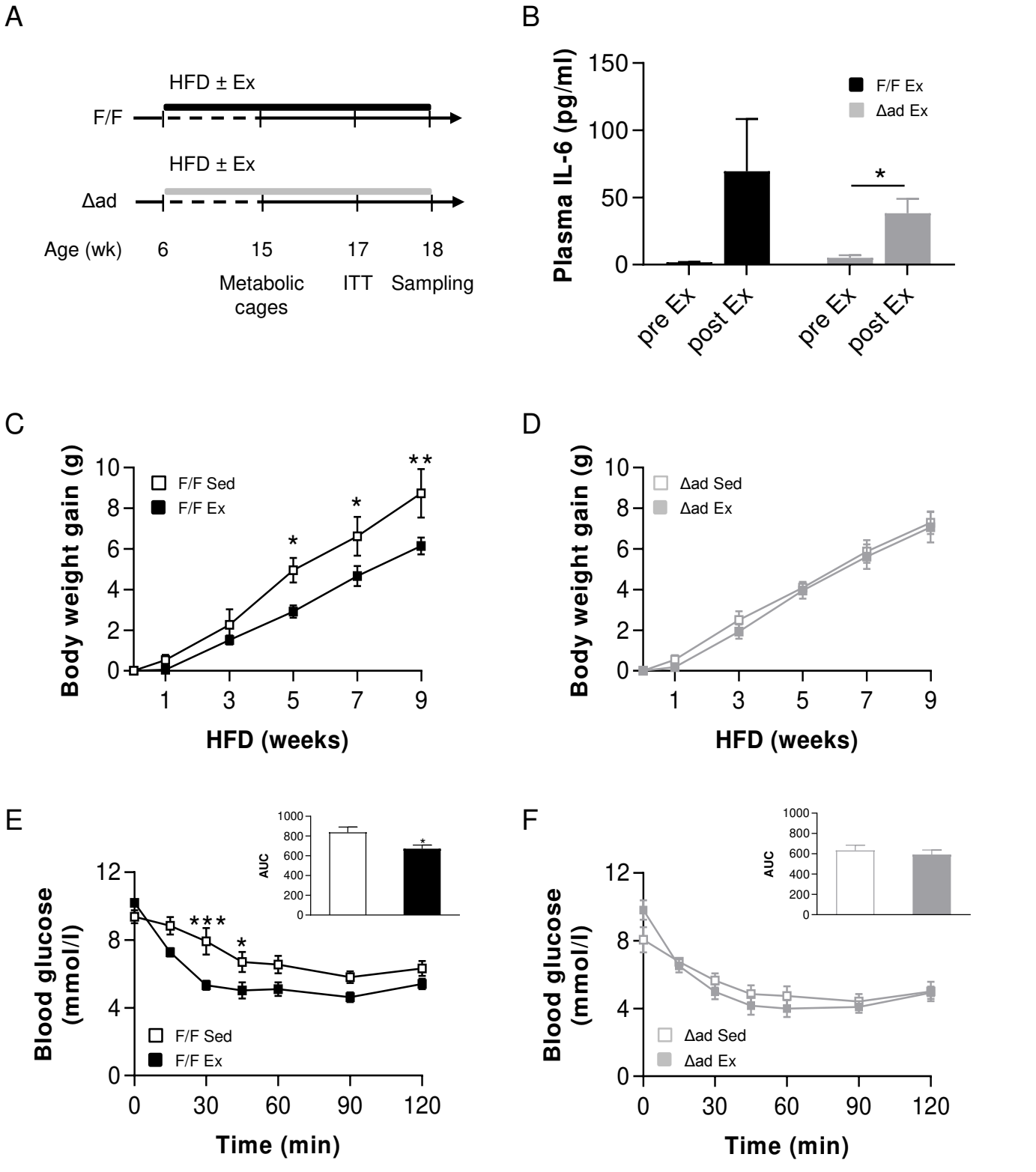
Figure 4 Improved leptin and insulin sensitivity in exercised HFD-fed gp130$^{F/F}$ mice

Circulating leptin (n=8) (**A**) and insulin (n=5-6) (**B**) levels were assessed in HFD-fed exercised gp130$^{F/F}$ and gp130$^{\Delta adipo}$ mice. Blood was sampled before a bout of exercise after 7 weeks of exercise training. (**C-F**) Representative Western blots and quantification of respective hypothalamic proteins in HFD-fed sedentary (Sed) (gp130$^{F/F}$ (n=5) and gp130$^{\Delta adipo}$ (n=6)) and exercised (Ex) (gp130$^{F/F}$ (n=7) and gp130$^{\Delta adipo}$ (n=6)) mice. Values are expressed as mean ± SEM. *p < 0.05, **p<0.01 (*t*-test). F/F: gp130$^{F/F}$; ∆ad: gp130$^{\Delta adipo}$.

Figure 5 Suggested model

In obesity, increased IL-6 levels contribute to elevated gp130-mediated leptin release from adipose tissue. Subsequently, leptin induces glucagon-like peptide-1 (GLP-1)-mediated insulin secretion from pancreatic β-cells (7). In turn, increased insulin and leptin signalling in the brain reduces food intake after repetitive physical activity. Such mechanism may be blunted in sedentary individuals due to HFD-induced central insulin/leptin resistance.

Figure 1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HFD (weeks)
D
Body weight gain (g)
10
8
6
4
2
0
Δad Sed
Δad Ex
1
3
5
7
9
HFD (weeks)
E
Blood glucose (mmol/l)
12
8
4
0
***
*
F/F Sed
F/F Ex
0
30
60
90
120
Time (min)
AUC
1000
800
600
400
200
0
*
F
Blood glucose (mmol/l)
12
8
4
0
Δad Sed
Δad Ex
0
30
60
90
120
Time (min)
AUC
1000
800
600
400
200
0

G

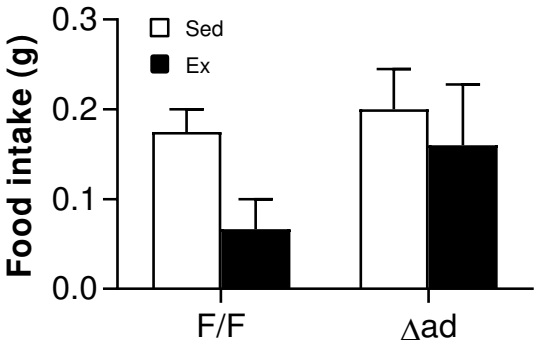
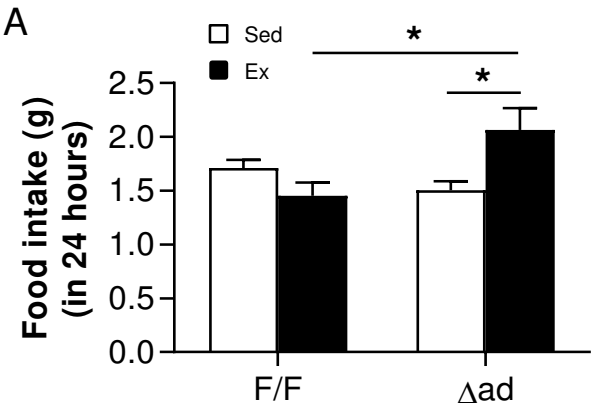
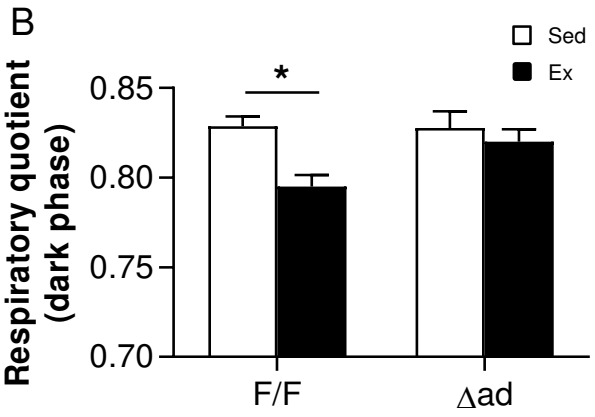


Figure 2


A
Sed
Ex
*
*
Food intake (g)
(in 24 hours)
2.5
2.0
1.5
1.0
0.5
0.0
F/F
Δad


B
Sed
Ex
*
Respiratory quotient
(dark phase)
0.85
0.80
0.75
0.70
F/F
Δad

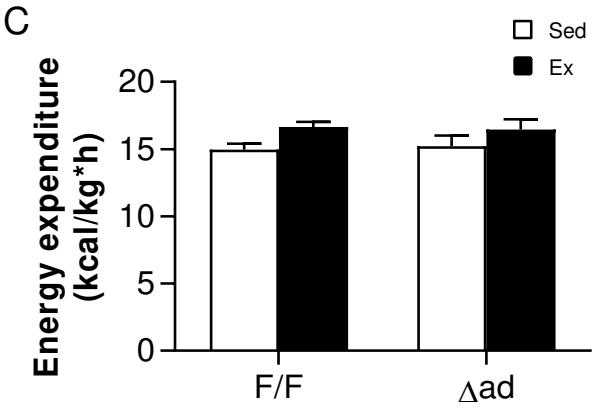
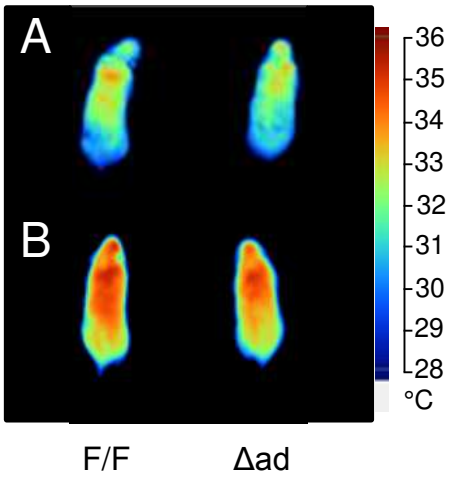
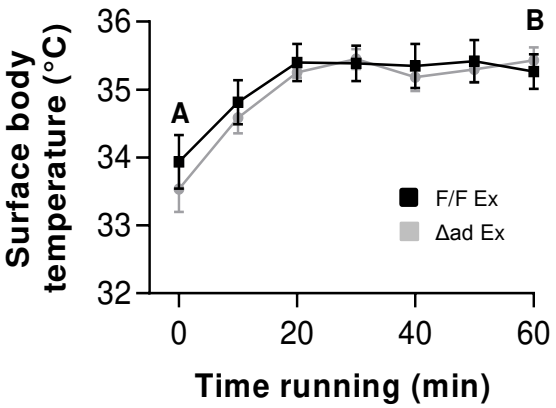

C
Sed
Ex
Energy expenditure
(kcal/kg*h)
20
15
10
5
0
F/F
Δad

Figure 3

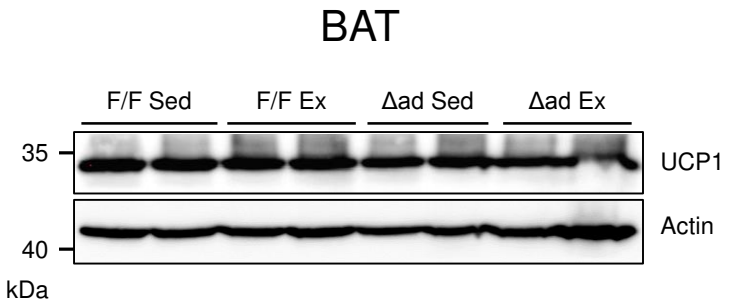
A


A
B
36
35
34
33
32
31
30
29
28
°C
F/F
Δad

B


Surface body temperature (°C)
36
35
34
33
32
A
B
F/F Ex
Δad Ex
0
20
40
60
Time running (min)

C


BAT
F/F Sed
F/F Ex
Δad Sed
Δad Ex
35
40
kDa
UCP1
Actin

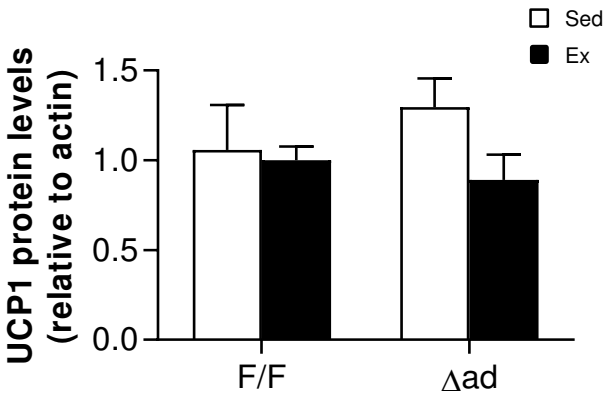

Sed
Ex
UCP1 protein levels (relative to actin)
1.5
1.0
0.5
0.0
F/F
Δad

Figure 4

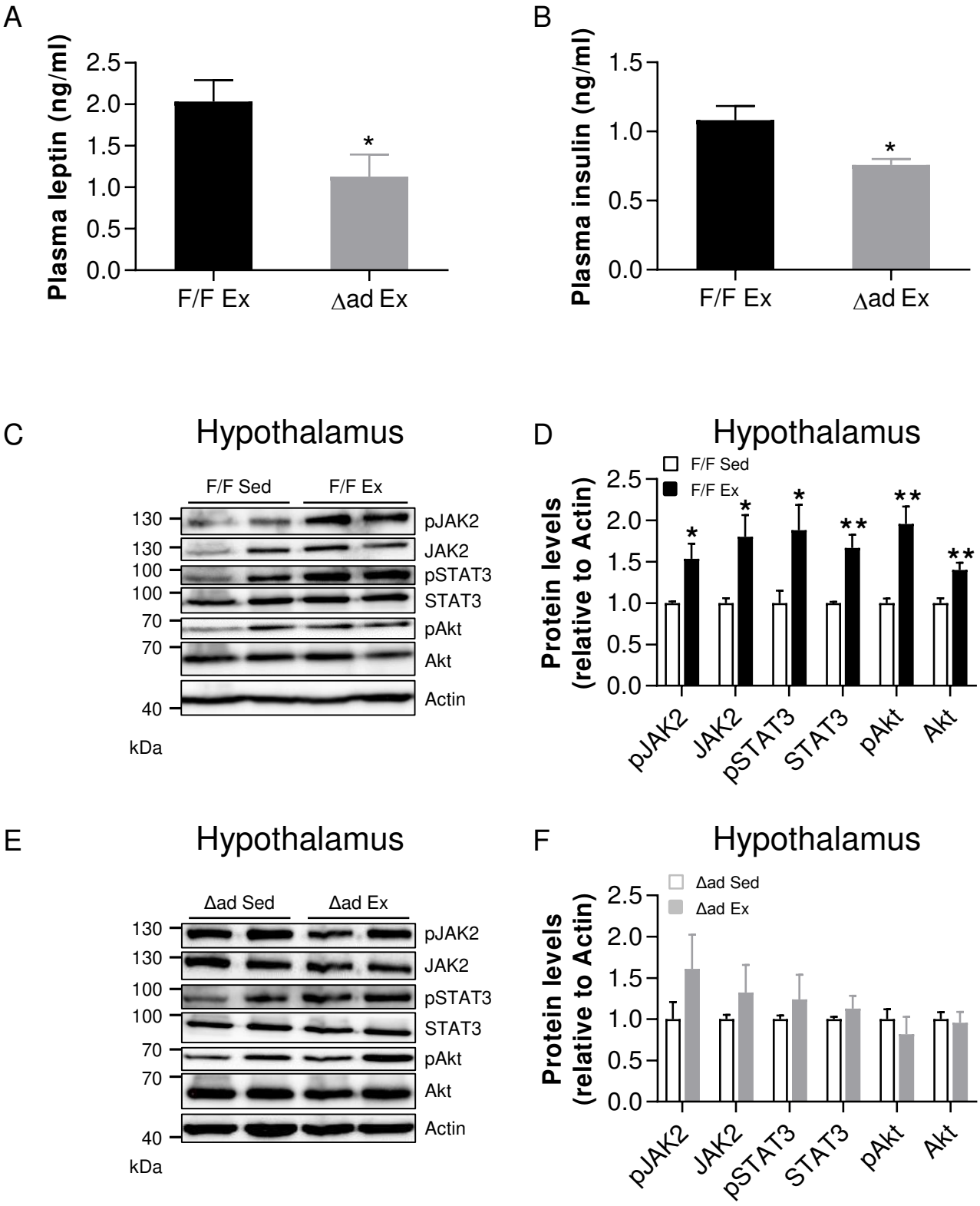
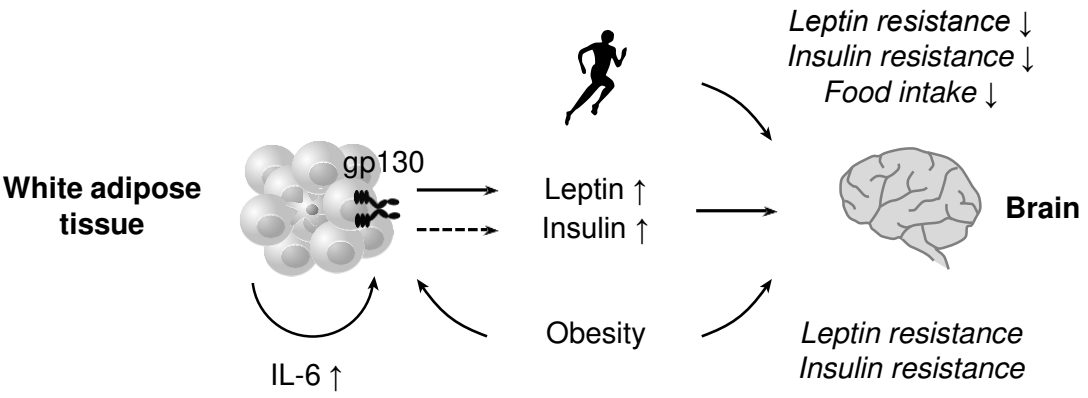

A
Plasma leptin (ng/ml)
2.5
2.0
1.5
1.0
0.5
0.0
*
F/F Ex
Δad Ex
B
Plasma insulin (ng/ml)
1.5
1.0
0.5
0.0
*
F/F Ex
Δad Ex
C
Hypothalamus
F/F Sed
F/F Ex
130
130
100
100
70
70
40
pJAK2
JAK2
pSTAT3
STAT3
pAkt
Akt
Actin
kDa
D
Hypothalamus
F/F Sed
F/F Ex
Protein levels (relative to Actin)
2.5
2.0
1.5
1.0
0.5
0.0
*
*
*
**
**
**
pJAK2
JAK2
pSTAT3
STAT3
pAkt
Akt
E
Hypothalamus
Δad Sed
Δad Ex
130
130
100
100
70
70
40
pJAK2
JAK2
pSTAT3
STAT3
pAkt
Akt
Actin
kDa
F
Hypothalamus
Δad Sed
Δad Ex
Protein levels (relative to Actin)
2.5
2.0
1.5
1.0
0.5
0.0
pJAK2
JAK2
pSTAT3
STAT3
pAkt
Akt

Figure 5


Leptin resistance ↓
Insulin resistance ↓
Food intake ↓
White adipose tissue
gp130
Leptin ↑
Insulin ↑
Brain
Obesity
Leptin resistance
Insulin resistance
IL-6 ↑